PHASE I SBIR MODEL APPLICATION

As part of a new web-based outreach effort that the NHLBI is implementing to improve the quality of our SBIR program, we have developed a model Phase I SBIR application for applicants to print and use as a guide for preparing their applications. We developed the model to address the needs of SBIR applicants who have limited grant writing experience and whose applications are often either returned without review due to improper format or fail in review due to the inadequate development of an excellent idea.

The Phase I model presented here was developed by a committee of NIH scientific program and review staff who have many years of experience in evaluating SBIR applications and advising applicants. The model is based on a recent Phase I application that received an outstanding priority score and was succeeded by an equally successful Phase II award. Under privacy requirements, the model has been altered to show fictitious investigator names, company, identifying numbers, budget, resources, bibliographies, and references. The textual sections of the application have not been significantly altered so that the model presents a true record of how the investigator presented a well thought out plan that succinctly articulates the scientific, technical and commercial merit of the idea.

This Phase I model is a supplemental aide for applicants. It is meant to be used in conjunction with the instructions contained in the "Omnibus Solicitation of the Public Health Service for SBIR Grant Applications" to which you can link on this home page. The model is organized according to the instructions for Phase I grant Application Form (PHS 6246-1) contained in the Omnibus Solicitation document. It demonstrates the correct type size, page lengths, and order of all the component parts of a Phase I application. It has highly legible text, charts, and graphics. There is a concise statement of the innovation under study. The narrative sections of the Research Plan are complete in thought and content and follow the suggested order from A. Specific Aims to F. Vertebrate Animals. Where certain items are not relevant to this particular application such as justifications for equipment, travel, and supplies; there are notes to direct you to the relevant instructions should the application require these components. Also, although vertebrate animals are not part of this application, we included a Section F. Vertebrate Animals, with notes on how to complete this component if it applies to your proposal. Also included are sample consultant letters which are found at the end of the model.

Another resource for applicants to refer to when preparing an application is the booklet, "Advice on SBIR and STTR Applications," which can also be linked to on this home page. Its provides advice, commentary, and value judgements in addition to objective information, rules, and regulations. The information presented in this booklet does not replace the instructions in the current SBIR Omnibus Solicitation but is meant to provide encouragement and mentoring to those applying for NIH small business grants.

As you review this model a final key point to make is that you appreciate the amount of detail that is provided in the Research Plan. Lack of detail is often a serious pitfall for an application. Many meritorious applications fail because investigators, concerned about confidentiality, do not provide the detail necessary for a proper evaluation. Applicants should understand that the contents of their application are treated as 'confidential material' and do not become public information without their involvement.

The SBIR Program is an essential part of the NHLBI mission and we enthusiastically encourage the submission of applications that are relevant to our programs. We hope that this Phase I SBIR Model Application will serve as a useful tool for applicants as you develop and format your own proposals.

OMB No. 0925-0195
Expiration Date 08/31/2001

Department of Health and Human Services
Public Health Service

Small Business Innovation Research Program

Phase I Grant Application

Follow instructions carefully

Leave blank --- for PHS use only.

Type	Activity	Number
Review Group		Formerly
Council Board *(Month, year)*		Date Received

1. TITLE OF APPLICATION *(Do not exceed 56 typewriter spaces)*
Development of a Lipoprotein Analyzer on Flow FFF

2. SOLICITATION NO. PHS 97-2

3. PRINCIPAL INVESTIGATOR ☐ New Investigator

3a. NAME *(Last, first, middle)*	3b. DEGREE(S)	3c. SOCIAL SECURITY NO.
Smithe, Jane E.	Ph.D.	

3d. POSITION TITLE
Director of Product Evaluation

3e. MAILING ADDRESS *(Street, city, state, zip code)*
Analy tical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449
BITNET/INTERNET Address: RSAL@oracle.com

3f. TELEPHONE AND FAX *(Area code, number, and extension)*
TEL: (508) 385-1111
FAX: (508) 385-2222

4. HUMAN SUBJECTS
☐ NO
☒ YES
4a. If "yes," Exemption no. or
IRB approval date
☐ Full IRB **or** ☐ Expedited Review
4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS
☒ NO
☐ YES
5a. If "Yes," IACUC approval date
5b. Animal welfare assurance no.

6. DATES OF PROJECT PERIOD
From: 11-1-1996 *Through:* 4-30-1997

7. COSTS REQUESTED
7a. Direct Costs $ 80,000.00
7b. Total Costs $ 118,720.00

8. PERFORMANCE SITES *(Organizations and addresses)*
Analytical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449

9. APPLICANT ORGANIZATION *(Name and address of applicant small business concern)*
Analytical Products, Inc.
2320 West St. , Suite 16
Salt Lake City, UT 84119-1449

10. ENTITY IDENTIFICATION NUMBER
1-058367978-A2
Congressional District 2

11. SMALL BUSINESS CERTIFICATION
☒ Small Business Concern ☐ Women-owned
☐ Socially and Economically Disadvantaged

12. NOTICE OF PROPRIETARY INFORMATION: The information identified by asterisks(*) on pages _____ of this application constitutes trade secrets or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that such information shall be used or disclosed only for evaluation of this application, provided that, if a grant is awarded as a result of or in connection with the submission of this application, the Government shall have the right to use or disclose the information herein to the extent provided by law. This restriction does not limit the Government's right to use the information if it is obtained without restriction from another source.

13. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title only of your proposed project, and the name, address, and telephone number of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information or possible investment?
☐ YES ☐ NO

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: John Jones, Ph.D.
Title: President
Address: Analytical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449
Telephone: (508) 385-3333
FAX: (508) 385-4444
BITNET/INTERNET Address:

15. PRINCIPAL INVESTIGATOR ASSURANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a *(In ink. "Per" signature not acceptable.)* DATE

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTABLE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF PERSON NAMED IN 14 *(In ink. "Per" signature not acceptable.)* DATE

Abstract of Research Plan

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

Analytical Products, Inc.

2320 West St., Suite 16

Salt Lake City, UT 84119-1449

(508) 385-1111

YEAR FIRM FOUNDED	NO. OF EMPLOYEES *(include all affiliates)*
1985	12

TITLE OF APPLICATION

Development of a Lipoprotein Analyzer Based on Flow FFF

KEY PERSONNEL ENGAGED ON PROJECT

NAME	ORGANIZATION	ROLE ON PROJECT
Jane E. Smithe, Ph.D.	Analytical Products, Inc.	Principal Investigator
John Jones, Ph.D.	Analytical Products, Inc.	Co-Investigator
Andrew Summer, B.S.	Analytical Products, Inc.	Engineer
Mary Yang, Ph.D.	Analytical Products, Inc.	Chemist
Charles Pierce, Ph.D.	University of Utah	Consultant
William Little, Ph.D.	University of Utah	Consultant

ABSTRACT OF RESEARCH PLAN: State the application's broad, long-term objectives and specific aims, making reference to the health-relatedness of the project. Describe concisely the research design and methods for achieving these goals and discuss the potential of the research for technological innovation. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. *Therefore, do not include proprietary or confidential information.* DO NOT EXCEED 200 WORDS.

Development of a Lipoprotein Analyzer system is proposed for rapid analysis of and characterization of the HDL, LDL, and VLDL profiles of human blood plasma. Preliminary results using a prototype system demonstrated the potential of the proposed system: the HDL, LDL, and VLDL fractions were clearly separtated, plasma samples were analyzed without sample pre-treatment, and differences in lipoprotein profiles were noted for different patients. Analyses times were between 20 minutes and one hour. Determination of accurate particle size of the lipoprotein complexes also demonstrated the unique capabilities of this system. The separation strategy of the proposed Lipoprotein Analyzer is based on Flow Field-Flow Fractionation, (Flow FFF), an elution based technique. This methodology is capable of high resolution measurements and is bio-compatible for analysis of biological macromolecules ranging from proteins up to chromosomes and cells. Thus this instrumentation is potentially a powerful and effective tool for rapid and direct measurement of the entire subfraction set of the lipoprotein profile. We propose to further study, optimize, and develop Flow FFF so as to ultimately generate a rugged, routine technique. We expect this development will provide a more expedient and less expensive method of lipoprotein characterization than currently available.

Provide key words (8 maximum) to identify the research or technology.

lipoproteins, plasma, serum cholesterol, HDL, LDL, VLDL characterization

Provide a brief summary of the potential commercial applications of the research.

The need for screening serum cholesterol levels is immense (see pages 19-20) and so there is a large market potential for the proposed Lipoprotein Analyzer. The proposed technique is expected to provide a rapid, low cost alternative to the current techniques. Also, the system could be used in research laboratories for direct determination of lipoprotein particle size and for semi-preparative fractionation of lipoprotein component.

Principal Investigator *(Last, first, middle):*

Budget for Phase I---Direct Costs Only

FROM 11-1-1996 TO 4-30-1997

PERSONNEL *(Applicant organization only)* NAME	Role on Project	Type Appt. *(months)*	% Effort on Project	Institutional Base Salary	DOLLAR AMOUNT REQUESTED *(omit cents)* Salary Requested	Fringe Benefits	TOTALS
Jane E. Smithe, Ph.D.	P.I.	12	60				
John Jones, Ph.D.	Co P.I.	12	30				
Andrew Summer, B.S.	Engineer	12	30				
Mary Yang, Ph.D.	Chemist	12	60				
SUBTOTALS →					46,424	10,000	56,424

CONSULTANT COSTS Charles Pierce, Ph.D., University of Utah William Little, Ph.D., University of Utah Ann Howard, Ph,D., University of Utah		3,000
EQUIPMENT *(Itemize)* (If over $15,000, itemize and justify all items over $5,000 on page 4 of the application)		2,400
SUPPLIES *(Itemize by category)* (If over $15,000, itemize and justify categories of items over $1,000 and itemize number and kind of animals, unit purchase cost, and unit care cost on page 4 of the application.)		14,976
TRAVEL (If over $5,000, itemize and justify on page 4.)		
PATIENT CARE COSTS	Inpatient	
	Outpatient	
CONTRACTUAL COSTS Machine shop (to be contracted out)		3,200
OTHER EXPENSES *(Itemize by category)* (if over $5,000, itemize and justify on page 4.)		
TOTAL DIRECT COSTS *(Also enter on Face Page, Item 7a)* →		$ 80,000
FIXED FEE REQUESTED		$ 6,720

OTHER SUPPORT *(see instructions)* ☒ NO ☐ YES

Budget Justification

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify costs accordingly.

PERSONNEL
Jane E. Smithe, Ph.D., P.I. - will supervise and coordinate the experimental studies, facilitate novel design, fabrication and testing.

John Jones, Ph.D., Co P.I. - will assist Dr. Smithe with these responsibilities.

Andrew Summer, Engineer - will assist in design and software development to streamline the operation.

Mary Yang, Chemist - will assist with the presentation of the plasma sample and the lipoprotein standards.

CONSULTANTS
Charles Pierce, Ph.D. - will assist in the interpretation of experimental data and the design and development of the commercial Lipoprotein Analyzer. Rate: $200 per day X 3 days.

William Little, Ph.D. - will assist in adapting the research version of the flow FFF apparatus into a more rugged, user-friendly commercial instrument. Rate: $200 per day X 7 days.

CONTRACTUAL COSTS
An outside machine shop will be required for fabrication of the flow FFF system out of the lucite blocks.

FIXED FEE
A fee of 6% of direct plus indirect costs ($80,000 + $32,000 = $112,000) is requested. This contributes to the capitalization of the company, and provides for expansion of resources and services.

Resources

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant small business concern and any other performance site listed on the FACE PAGE. Identify support services such as secretarial, machine shop, electronics shop, and the extent to which they will be available to the project. Use continuation page(s) if necessary.

Analytical Product's laboratories are located in Valley City, a business/ industrial area of Salt Lake City, Ut. At this location, we are a 20 minute drive from the University of Utah where Drs. Pierce and Little have their offices. Our laboratory occupies 2,000 square feet. For research and development purposes, we have several FFF systems, computers, and pumps set up permanently including sedimentation, thermal, and flow FFF systems. As an instrumentation manufacturer, we also have a flux of FFF systems through our lab.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

3 four-channel pumping systems that provide 4 independent constant flow rates for multi-channel operation
2 UV/Vis detectors including one multi-wavelength UV detector
1 ERMA refractive index detector
3 single-channel flow FFF channel systems

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

Jane E. Smithe — Director of Product Evaluation, Analytical Products, Inc.

Education

Institution and Location	Degree	Date Conferred	Field of Study
Texas Baptist College, Dallas, TX	B.S.	1979	Chemistry
University of Utah, Salt Lake City, UT	Ph.D.	1987	Phys./Anal Chem.

Research and Professional Experience

1990 - present — Director of Product Evaluation, Analytical Products, Inc., Salt Lake City, Utah. Responsibilities include sales and customer support through analysis of their samples and participation in new product development. Author and principal investigator of one NSF SBIR and two NIH SBIR grants Invited speaker at the Second and Third International FFF Symposium held in Salt Lake City and Park City, Utah respectively.

1987 - 1990 — Senior Chemist, Procter and Gamble, Soaps and Detergents Division, Cincinnati, OH. Responsible for particle characterization group within the Analytical Section of the Soaps and Detergents Division. Supervised the microscopy group as well as partial responsibility in the HPLC group. Honors included invited speaker for 17th Annual Analytical Symposium of Procter and Gamble. Also was awarded one year's free use of the DuPont SF 2000 Sedimentation Field-Flow Fractionator based on a proposal submitted to DuPont for the analysis of clays and the rheological effects of the particle size distribution.

1979 - 1987 — Research and/or Teaching Assistant, University of Utah. Assisted in general chemistry, physical chemistry, environmental chemistry, quantitative analysis, and instrumental analysis classes. Research was in Charles Pierce's group studying the application of sedimentation FFF to the analysis of river water particles. Honors include Stauffer Chemical Company Award, 1979; University of Utah Honor Scholarship, 1979-1980.

Publications

J.E. Smithe and D.C. Price, "Optimization Study of Octane-in-Water Emulsions by Field-Flow Fractionation", J. Chromatogr., 317, 433-444 (1992).
J.E. Smithe and C. Pierce, "Retention Perturbations Due to Particle Interactions in Sedimentation Field-Flow Fractionation", J. of. Chem., 65, 211-219 (1992).
J.E. Smithe and C. Pierce, and R. Becker, "Sedimentation FFF VI Combined with Perturbations Due to Electrostatic Repulsion", J. Colloid Interface Sci., 132, 300-312 (1994).
J.E. Smithe, C. Pierce, and R. Becker, "Secondary Relaxation in Programmed Field-Flow Fractionation", Anal. Chem., 41, 1234-1242 (1995).

Manuscripts in Preparation

J.E. Smith, and C. Pierce, "Methods to Void Volumes in FFF Channels."
J.E. Smith and G. Lu, "Analysis of Colloidal Silica by FFF."

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

John Jones — President and C.E.O., Analytical Products, Inc.

Education

Institution and Location	Degree	Date Conferred	Field of Study
University of Wisconsin	B.S.	1967	Chemistry
University of Utah	Ph.D.	1973	Physical Chem.

Research and Professional Experience

1988 - present — President and C.E.O., Analytical Products, Inc., Salt Lake City, Utah. Involved in research, development, and sales of fractionations systems.

1986 - 1988 — Director of Technology, Ionex Corporation, Sunnyvale, CA. Named editorial advisor, Journal of Fractionations, member of the scientific organization committee, International Fractionation Symposium, editorial advisor Journal of CL/CG, member of the editorial board, Journal of Liquid Chromatography. Session Chiarman, Beijing Conference and Exhibitions on Instrumental Analysis, Beijing, China.

1986 - 1988 — Vice President of Research and Engineering, Key Scientific, Inc., Salt Lake City, Utah. Session chairman, Eastern Analytical Symposium, 1986 and 1987.

1983 - 1986 — Manager, Chromatography System Research and Development, Lee Associates, Walnut Creek, CA. Session chairman, Eastern Analytical Symposium, 1984 - 1985.

1976 - 1983 — Senior Research Chemist, Department of Research and Engineering, Lee Associates, Walnut Creek, CA.

1975 - 1976 — Postdoctoral Research Associate, Department of Chemistry, University of Utah.

1973 - 1975 — Postdoctoral Research Associate, Department of Chemistry, Oregon State University

Publications

Gas Chromatography with Open Tubular Column, John Wiles Publishers, New York, 1984.
Author of 14 publications regarding field-flow fractionation. Two pertinant articles are:
J. Jones, "Flow Field-Flow Fractionation Techniques", Anal. Chem., 62, 1212-1234 (1994).
J. Jones, W. Little, " Flow FFF/Filtration System for Bioproducts Analysis", J. Chromatogr., 715, 344-354, (1995)
Also holds 10 patents regarding chromatographic techniques or instruments.

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

Andrew Summer — Staff Engineer, Analytical Products, Inc.

Education Institution and Location	Degree	Date Conferred	Field of Study
San Diego State University	B.S.	1988	Physics

Research and Professional Experience

1991 - present — Production Engineer, Analytical Products, Inc., Salt Lake City, Utah. Responsibilities include design of new and/or improved FFF instrumentation as well as purchasing and production for commercial field-flow fractionation instrumentation, development of new suppliers and interfacing with computer consultants. Supervision and training responsibilities for one technician. Co-author of several FFF presentations.

1989 - 1991 — Technician, Analytical Products, Inc., Salt Lake City, Utah. Responsibilities included construction of both mechanical and electrical systems of FFF instrumentation and sample analysis.

Summer 1986 — Research associate involved in study of ecological impact study of Alaska north slope tundra. Co-author of resulting publication.

Mary Yang — Chemist, Analytical Products, Inc.

Education Institution and Location	Degree	Date Conferred	Field of Study
Beijing University	B.S.	1982	Chemistry
Lanzhou Inst. of Chemical Physics	Ph.D.	1989	Anal./Photchem.

Research and Professional Experience

1991 - present — Senior Researcher at Field-Flow Fractionation Research Center, Department of Chemistry, University of Utah. Studies of biological separation and characterization, and flow field-flow fractionation instrument development.

1990 - 1991 — Research Associate of Lanzhou Institute of Chemical Physics, Chinese Academy of Science. Emphasis on HPLC and capillary electrophoresis separation of biological materials.

1984 - 1990 — Research Assistant of Lanzhou Institute of Glaciology and Geocryology, Chinese Academy of Science. Emphasis on GC, GC-MS of hydrogen and oxygen isotopes and ion chromatography. Awarded outstanding Ph.D. Student by President of Chinese Academy of Science, Beijing.

Publications

Yang, M., "Bioseparation Mechanics", Instrument Guide, 4, 25-35, Beijing Press, 1994.
Min Liu, M. Yang, and Charles Pierce, "Rapid Protein Separation and Diffusion Coefficient Measurement by Flow FFF", J. of Protein Analysis., 1, 1623 (1995).
C. Pierce, M.A. Bennett, M. Liu, and M. Yang, "Techniques to Separate Polymers and Biological Materials.", J. of Protein Analysis, 3, 1230 (1996).

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

Charles Pierce — Distinguished Professor, University of Utah

Education		**Date**	
Institution and Location	**Degree**	**Conferred**	**Field of Study**
Brigham Young University	B.S.	1952	Chemistry
University of Utah	Ph.D.	1954	Physical Chem.

Research and Professional Experience

1989 - present — Distinguished Professor of Chemistry, University of Utah. A major area of his research has been chromatography in almost all of its fundamental aspects. He has also worked on the unification of separation theory, new separation methodology, macromolecular separations, techniques for diffusion coefficient measurements, theory of diffusion, chemical kinetics, and snow and avalanche physics. He has invented and extensively developed the versatile field flow fractionation method for macromolecular separations. He has been active in research and education dealing with environmental problems.

1966 - 1989 — Professor of Chemistry., University of Utah.
1962 - 1966 — Research Professor of Chemistry, University of Utah
1959 - 1962 — Associate Professor of Chemistry, University of Utah
1957 - 1959 — Assistant Professor of Chemistry, University of Utah

Awards:
ACS Award on Chromatography and Electronics, 1967
Nebraska Lectureship Award, 1969
Utah Award, Local Section of American Chemical Society, 1970
ROMCOE Award, Outstanding Environmental Achievement, 1973
Fulbright Grant, Cayetano Heredia University, Lima, Peru, 1974
Stephen Del Nogare Chromatography Award, 1979
Russian Scientific Council Chromatography Award, 1980
ACS Award in Separations Science and Technology, 1986

Publications

Author of over 365 publications. Three articles relevant to this project are:

C, Pierce, “Chromatography Dynamics”, J. Chromatogr., 600, 200-220 (1994).
C. Pierce, “Chemistry and Flow Dynamics”, Anal Chem., 72, 1211-1230 (1995).
C. Pierce, “Fractionation Science”, Anal Chem., 80, 1300-1315 (1995).

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

William Little — Professor of Chemistry
University of Utah

Education

Institution and Location	Degree	Date Conferred	Field of Study
Brigham Young University	B.S.	1950	Chemistry
Brigham Young University	M.S.	1952	Organic Chem.
University of Utah	Ph.D.	1965	Physical Chem.

Research and Professional Experience

1990 - present	Professor of Chemistry, University of Utah.
1986 - 1990	Vice President of Research and Development, Analytical Products, Inc. Salt Lake City, Utah.
1978 - 1986	Associate Research Professor, Dept. of Chemistry, University of Utah. Responsibilities include design and engineering of new and improved field-flow fractionation, detector, and pump instrumentation.
1967 - 1978	Assistant Research Professor and Instructor, Department of Chemistry, University of Utah.
1965 - 1967	Postdoctoral Research Associate and Instructor, Department of Chemistry, University of Utah.
1961 - 1962	Senior Chemist, General Aircraft, Co., Pleasanton, CA.
1957 - 1961	Senior Chemist, General Aircraft Co., Aircraft Propulson Division, Iowa City, Iowa.
1951 - 1957	General Aircraft Co., Chemist, Richland, WA.

Professional Societies and Honors

Member, Editorial Board of Separation Science, published by Martin Drake.
Chairman of Winter Student Meeting of Salt Lake City anc Central Utah Sections of Amercian Chemical Society, January 1973, University of Utah.
Phi Eta Sigma, Freshman Honor Society; Phi Kappa Phi; Sigma XI, American Chemical Society

Invited Talks

Society for Applied Spectroscopy, 1986, Chicago.
ACS National Meeting April 1985, Dallas, Texas.
ACS Symposium on Separations Science & Technology, 1984, New York.

Publications

Co-author of 100 publications. Three relevant publications are listed below:
W. Little, J. Goodman, "Biological Separation Techniques", Protein Science., 5, 1530 (1993).
W. Little, K. Wright, "Diffusion Coefficient Measurement", Anal. Chem., 70, 711-719 (1994).
W. Little, R. Breen, "Flow FFF and Protein Separation, J. Chromatogr., 614, 222-244, (1996)

RESEARCH PLAN

A. Specific Aims

The innovative use of Flow FFF technology is proposed for the routine analysis of lipoproteins. Preliminary analysis of this class of samples has already demonstrated the potential of this technique for separation of the high density, low density, and very low density fractions of lipoprotein samples (Figure 1) (1). We propose to further study, optimize, and develop flow FFF, so as to ultimately generate a rugged, routine technique. We expect this development will provide a more expedient and less expensive method for lipoprotein characterization than currently available. Additionally, the characterization based on size will provide new database information which may be used for diagnostic purposes.

Correlation of Lipoprotein Properties with Incidence of Disease

Development of a new analytical method for characterization of lipoproteins is significant due to the strong correlation of coronary heart and artery disease with the presence of the various forms of this class of bio-macromolecules. Lipoproteins are basically complexes of lipids and proteins. The MW of the complexes is very large, in the range of 300,000 to 10,000,000 Daltons (2) so that the particle size of the complex is often a more appropriate description. These complexes vary in size, density, net charge, and apolipoprotein content. Eight types of apolipoproteins have been isolated and characterized: apo A-I, A-II, B, C-II, C-III, D and E (3), (4). The regulation of entry and exit of particular lipids at specific targets is coded by these protein components.

Traditionally lipoproteins have been classified and studied according to their density properties through ultra-centrifugal analyses. In general, low density lipoproteins (LDLs) are most strongly correlated with causality of coronary heart disease; the high density fraction (HDLs) have been linked to protection against the disease. More in-depth studies have found subfractions of these major groups as being stronger indicators of the risk of the disease (5). Recently, the apolipoprotein content has been recognized as a significant factor due to its role in the formation, structural stability, and in the metabolism of lipoproteins (4). However, the basic density-classed fractions are heterogeneous with respect to their apolipoprotein content, indicating that a sub-fraction of the density based fractions could be more significantly related to incidence of disease. Possibly studies using new separation strategies based on properties other than lipoprotein density could provide new and stronger correlating factors.

Competing Techniques

While ultracentrifugation is the reference research technique for studying lipoproteins and has many advantages for preparative scale isolation of the lipoprotein fractions, there are drawbacks involved with this technique. The monetary and time cost for ultracentrifugation analysis prohibits routine application in clinical work. Compositional changes of the lipoprotein complex may be induced due to shearing effects and ionic-strength forces (6,7).

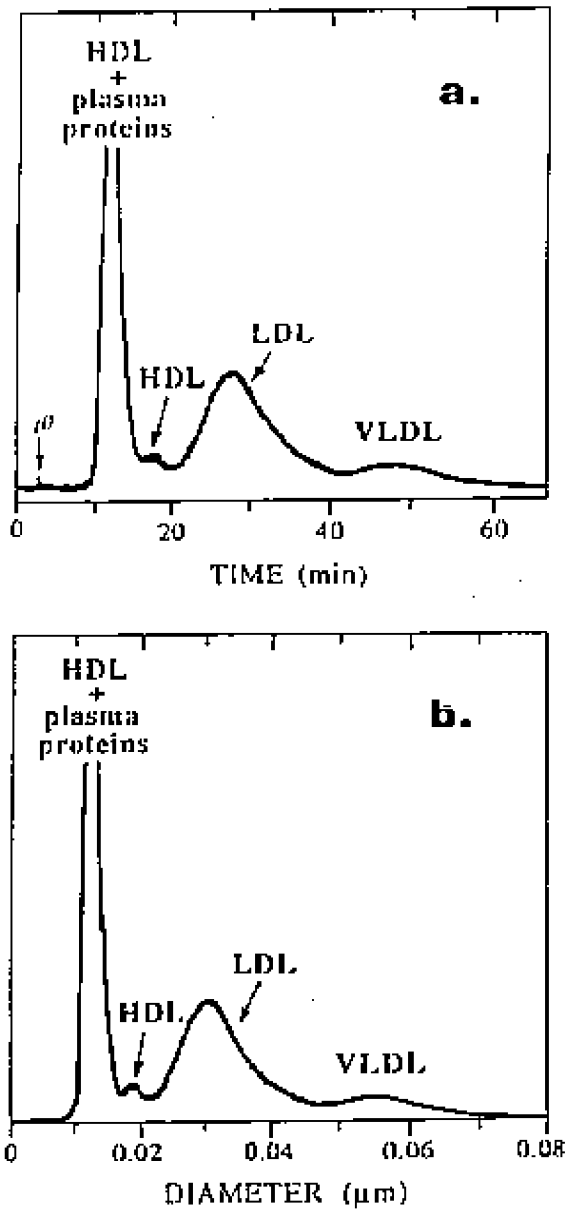

Figure 1. Analysis of proteins and lipoproteins using prototype Lipoprotein Analyzer. Carrier is phosphate buffer saline at pH 7.4, Vch = 1.1 mL/min, Vx = 6.9 mL/min, detection @280 nm.

In clinical labs lipoprotein determination procedures involve enzymatic and precipitation techniques. The accepted clinical procedure for lipid profile testing is summarized below:

Step 1. Plasma sample preparations: The blood sample is centrifuged for 10 minutes to separate cells from plasma. The centrifuge, such as the Beckman J-6M, costs about $11,000.

Step 2. Total cholesterol (TC) and total triglycerides (TG) determination: Total cholesterol and triglycerides in the plasma are determined by an automated TC analyzer using an enzymatic reaction method. The automated TC analyzer, such as the Hitachi 717, costs about $140,000. This procedure takes 10 minutes.

Step 3. High density lipoprotein cholesterol (HDL-C) determination: HDL-C is separated from other lipoproteins by precipitating out low density lipoprotein cholesterol from plasma using 50 Dka dextran sulfate and magnesium chloride. The HDL-C is measured from the clear supernatant by the automated analyzer using the enzymatic method noted in step two. This procedure takes another 10 minutes.

Step 4. LDL-C and VLDL-C estimation: VLDL-C (very low density lipoproteins) are determined indirectly using the following formula:

$$VLDL = TGL/5$$

$$LDL = TC - (HDL - C) - (VLDL - C)$$

The above procedures for lipid profile analysis cost about $20 per sample excluding instrument maintenance costs and depreciation. Although this procedure has been commonly used for clinical measurement, there are serious drawbacks. One important consideration is the inaccuracy and incompleteness, as the calculations required assumptions and are indirect. Large relative inaccuracies have been reported for the TC measurement using the enzymatic reaction (step 2) (8). This procedure also assumes that the triglyceride level is highly correlated with VLDL-C level and requires plasma sample from fasting individuals. Additionally, poor reproducibility of the precipitation reaction (step 3) has led to larger analytical errors: coefficients of variation in the range of 5% to 38% (9). A last disadvantage is that the subspecies of the HDL, LDL, and VLDL fractions cannot be determined.

Other Competing Techniques

Numerous other analytical methods have been alternatively explored which exploit properties other than density. These methods, including electrophoretic and chromatographic techniques, are either time consuming, provide poor resolution, are restricted to low molecular weight species (less than 10 Daltons), or are difficult to quantify.

The state-of-the-art chromatographic technologies include commonly used techniques such as high pressure liquid chromatography, gel permeation chromatography, affinity chromatography, and ion chromatography. These extensively developed techniques have limited applicable molecular weight ranges (approximately 10 daltons) and suffer from many problems such as sample loss due to surface adsorption, degradation of biological activity due to their interaction with either organic solvents or the active reaction sites on the packed bed. Nevertheless, an affinity chromatographic method using heparin-sepharose columns has been used to separate the lipoproteins of a particular density class by apoliprotein content (10).

High resolution electrophoretic techniques are commonly used for peptide, amino acids, and DNA sequencer applications. However, this technique has the disadvantage of limited applicability to small molecules only. Quantitation of the lipoprotein fractions has been a major difficulty of this technique. Also, adsorption loss or peak shape distortions are often observed for proteins, DNA, etc., especially in fused silica capillary zone electrophoresis. The proposed technique, flow FFF, is a rapid, high resolution, open channel separation technique with great flexibility towards carrier solvent pH and ionic strength. These features indicate the potential for routine analysis of lipoprotein. In addition, due to the high resolution capabilities of this technique the possibility exists for characterizing the sub-species of the lipoprotein fractions.

B. Significance

Principles of the Proposed Lipoprotein Analyzer

For perspective on the developmental needs of the flow FFF technology towards generation of a routine technique for lipoprotein analysis, a brief description of the general FFF technique and flow FFF follows. More details can be found in references 1 and 11.

The basic technology of field-flow fractionation was developed in the late ‘60's. The first successful separation was accomplished in 1967 using a thermal gradient as the separation force; the technique was named Thermal FFF. Since then, the principles of FFF have extended to generate other sub-techniques of FFF: sedimentation and flow FFF. These later separation methods depend on a centrifugal field and a cross-flow field, respectively, as the driving force for separation.

In each FFF sub-technique, the field is applied perpendicular to a thin parabolic profile due to the geometric design of the FFF channel (Figure 2). The field differentially interacts with components of the sample so that zones of a characteristic thickness are generated in the flow channel. When laminar flow is initiated through the channel, the zones migrate at a rate determined by the zone thickness. Thus the combination of field and laminar flow generates differential retention and separation. Under normal mode retention conditions, the sample is fractionated in order of increasing molecular weight, since the characteristic zone thickness is, in general, related to the molecular weight of the sample species.

Flow FFF (Figures 3,4) is applicable to both polymers and particles. This technique provides high resolution separation of polymers, including biological macromolecules and particles in the size range of 3 nm (or 1000 Daltons) to 100 microns. The operating field for flow FFF is a cross-flow field which “drags” the macromolecules and particles to the accumulation wall due to viscous drag. Each sample species then diffuses into an equilibrium based zone of a specific thickness, as determined by its diffusive properties which in turn may be related to molecular weight or size. Axial channel flow through the channel has a parabolic profile which provides the means of differentiation. As the axial flow transports the zones along the accumulation wall through the channel, the zones become separated because of their differences in zone thickness.

Elution or retention time is thus governed by the diffusive properties which determine the thickness of the zone. Smaller particles or macromolecules elute before the larger as shown in Figure 3. Additionally, the cross-field strongly influences the elution time and may be used to “fine-tune” the separation to achieve the resolution or analysis speed desired. With knowledge of

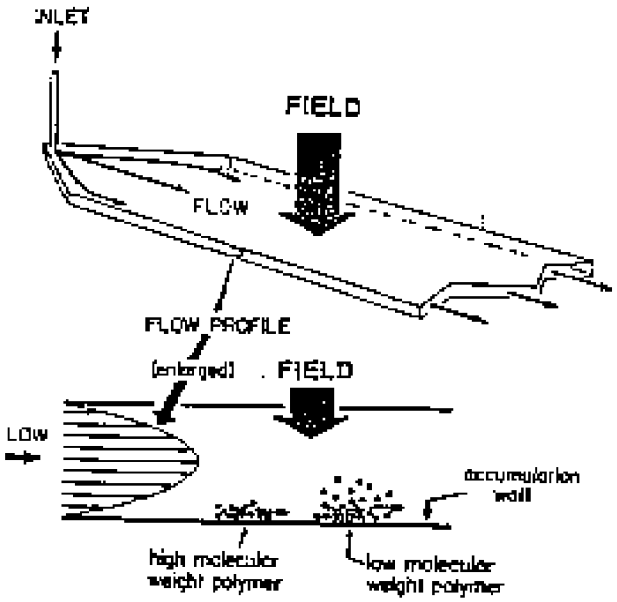

Figure 2. Schematic of basic FFF process. An external field is applied perpendicular to the length of a thin, ribbonlike channel.

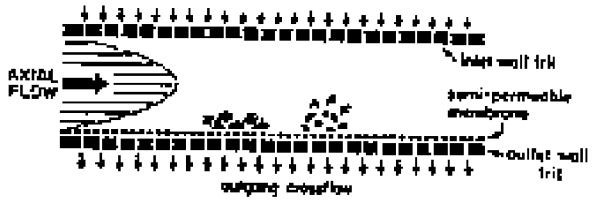

Figure 3. Schematic of separation in a Flow FFF channel.

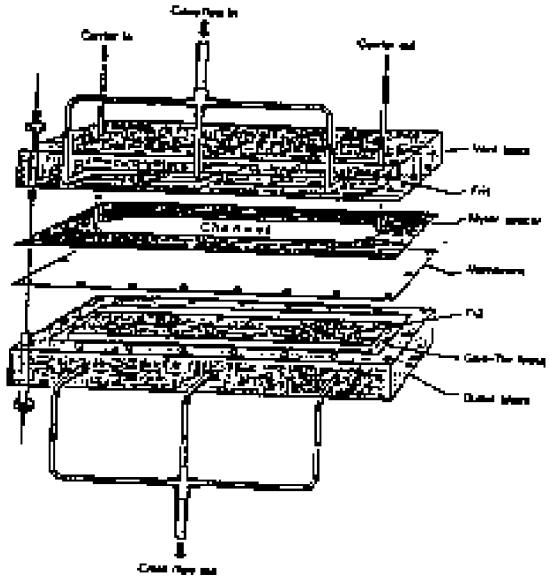

Figure 4. Design of Flow FFF channel

the magnitude of the cross-flow field and the channel flow rate, the elution profile may be mathematically manipulated as shown in the following equation to generate a particle size or MW distribution.

$$T_r = (\pi\eta\ w\ V_x) / 2\ k\ T\ V_{ch}\ d)$$

Where t_r is the retention time, η is the cross flow rate, k is Boltzmann's constant, T is the temperature, V_{ch} is the flow rate through the channel, and d is the Stokes diameter. For further optimization of analysis of a broad range of particle size, field programming may be used. In this case, the crossflow rate is initially high for better resolution of the small components. Following a period of constant high cross flow field, the cross flow pump is time programmed to decrease over the analysis period. The decrease in field strength accelerates elution of the larger components and so optimizes analysis time.

The instrument design shown in Figure 4 illustrates the simplicity of this technique. Cross flow is generated by pumping carrier fluid into the top reservoir, across the channel defined by a plastic spacer, through the membrane and out the bottom outlet block. A ceramic frit inserted in both the top block and bottom block distributes the flow into a finely divided viscous force. The membrane placed on the surface of the outlet block serves as the accumulation wall of the channel, allowing cross flow to permeate while retaining the sample components in the channel. Thus the membrane must be mechanically strong, smooth, and permeable.

Advantages of Flow FFF for Lipoprotein Analysis

The general advantages of flow FFF for biological applications such as lipoproteins are:

1. The flow FFF system is simple due to instrument design.
2. Flow FFF is a universally applicable separation process; the separation principle simply relies on the diffusion coefficient of the sample species. Thus separation of complex sample mixtures including bio-polymers and particles is possible.
3. The elution based principle of flow FFF facilitates the ease of automation and continuous fractionation, characterization, and preparative sample collection of size-classed fractions for further analysis, e.g. for analysis of apolipoprotein content.
4. The applied cross-flow field which controls separation is a physical and not a chemical force and thus facilitates ease of control and flexibility for "tuning" the separation conditions.
5. The materials used to construct the flow FFF apparatus may be optimized to allow for a wide range of carrier chemical conditions. Maximum compatibility of the carrier with the bio-polymer of interest may be achieved and the viability of the bio-polymer is preserved.
6. No additives such as densifiers, polymers, or electrolytes are required, minimizing the risk of biological activity loss or degradation. The size classed fractions produced are "clean" lacking these added contaminants.
7. High speed separation of bio-polymers or particles can be achieved by alteration of the elution flow velocity. Rapid purification of large amounts of biological samples is thus a distinct and feasible possibility.
8. As compared to chromatographic techniques, the technique is gentle. The samples experience none of the shear forces that exist in migration through the packed beds of a chromatographic column.

Preliminary Flow FFF Results for Lipoprotein

Initial studies as shown in Figure 1 and the following Figures 5 - 7 have been performed to demonstrate the potential of flow FFF for lipoprotein analyses and to map out the difficulties of this project.

Sample Preparation Techniques: Plasma, HDL, LDL, and VLDL samples were prepared as follows: Plasma samples were obtained by collecting blood using an evacuated blood collection tube containing dry disodium EDTA (1mg/mL) after a 12h fast and the cells were spun out by centrifuging about 30 minutes at 3000 rpm. The plasma (p = 1.006 g/mL) was ultracentrifuged (40,000 rpm 15^{o} C) for 24 h. The centrifugal tube was sliced to separated VLDL (the top fraction) from the bottom fraction. The density of the bottom fraction was adjusted to 1.063 g/mL using sodium bromide and the resulting solution was ultracentrifuged for another 24h. The LDL fraction was sliced from the top of the tube. The remaining HDL fraction was then separated by spinning 24h after adjusting the density to 1.21 g/mL.

Experimental Conditions: A flow FFF system similar to that shown in Figure 4 with channel dimensions: 28.5 cm in length by 2.0 cm breadth 〉 0.0178 cm thickness was used for both isocratic and programmed field experiments. The membrane used in the channel was a regenerated cellulose with a nominal MW cutoff of 30,000 Daltons. Carrier solution used both for the cross flow and the channel flow was phosphate buffer saline at pH = 7.4. Sample injection amounts were typically 10 uL. HPLC type pumps were used to deliver the channel and cross flow and HPLC type UV detector set @ 280 nm was used to monitor the separation.

Results and Discussion: Individual HDL, LDL, and VLDL fractions were analyzed separately to characterize retention behavior. Figure 5 shows the retention characteristics of these components under channel and cross flow conditions of 2.2 and 5.0 mL/min, respectively. The overlap of components in the HDL and LDL fractions and the LDL and the VLDL fractions indicates that either these fractions are not pure or that particles of similar size exist in different density fractions. However, the major components of each fraction were sufficiently separated. Adjustment of the channel and cross flow rate values for slightly higher resolution conditions were made and these conditions (V_{ch} = 1.1 mL/min, V_x = 6.9 mL/min) were applied to a blood plasma samples of three different patients. The resulting fractograms and calculated size distribution shown in Figure 6 demonstrate the potential of flow FFF for profiling lipoprotein content. The differences noted (which have not yet been conclusively interpreted) include the following: 1) an apparent difference in particle size of the VLDL, and LDL components, 2) appearance of an additional HDL component possibly HDL, for patient #001, and 3) appearance of a possible subfraction of LDL for patient #016.

In an attempt to achieve yet higher resolution without excessive analysis time, programmed field conditions were used. The cross flow pump was manipulated so that the initial pump rate was 9.0 mL/min. This crossflow field was then decreased after a period of about 10 minutes by gradually slowing the cross flow pump to 1.0 mL/min. This procedure allowed for better resolution of the HDL components as shown in Figure 7. The HDL_2 and HDL_3 components were separated. The corresponding plasma analysis however did not apparently contain any HDL_3 components. Better resolution of the LDL and VLDL was not achieved. Possibly these components have broad, overlapping size distributions. Alternatively, experimental conditions targeted at these components only could completely resolve the peaks.

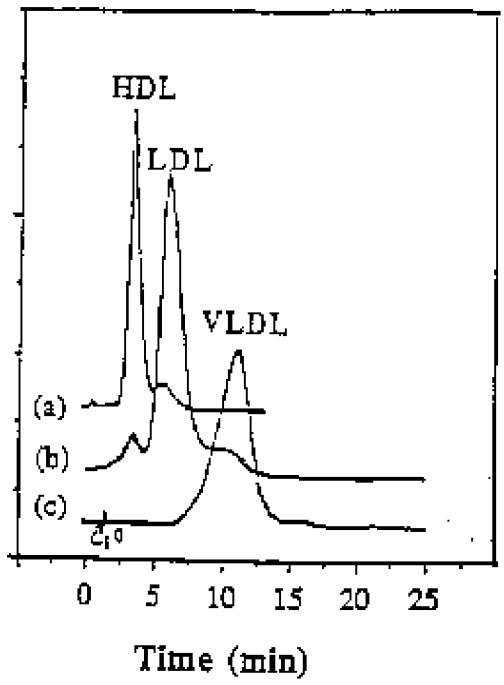

Figure 5. Separation of individual lipoprotein components.
(a) HDL fraction, (b) LBL fraction, (c) VLDL fraction
Vch = 2.2 mL/min, Vx = 5.0 mL/min, phosphate buffer saline.

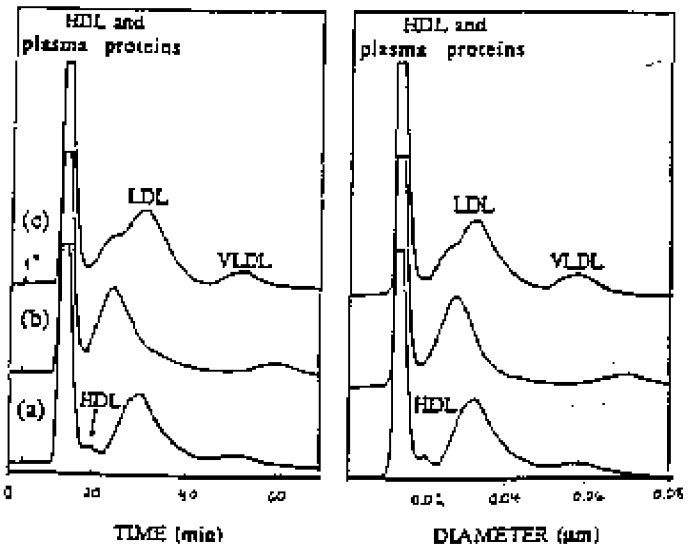

Figure 6. Separation of lipoproteins in human plasma.
(a) patient #001, (b) patient #002, (c) patient #018
Vch = 1.1 mL/min, Vx = 6.9 mL/min

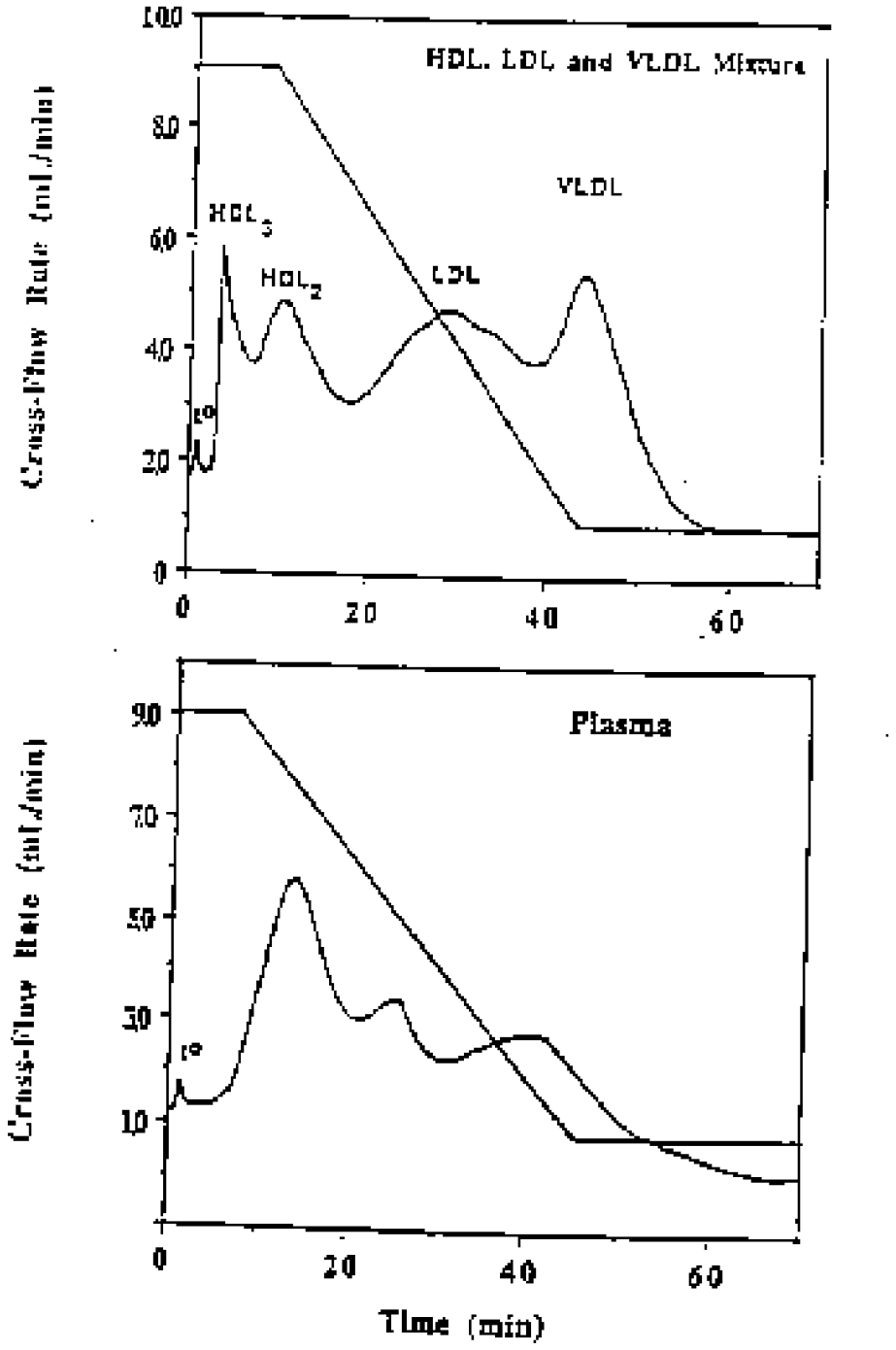

Figure 7. Programmed field separation of Human Plasma lipoproteins. Initial Vx = 9.0 mL/min, final Vx = 1.0 mL/min

The results above demonstrate the potential of flow FFF for lipoproteins for basic separation and profiling of lipoprotein content. The potential for accurate particle size determination of lipoproteins was additionally investigated by examining the retention results for the standard fractions. Stokes diameters were calculated using the standard flow FFF retention equation listed on page 15. Table 1. Shows the good agreement between these calculations and literature values.

Conclusions: Preliminary analyses indicated that flow FFF could be successfully applied to the analysis of lipoproteins. Capabilities for separation and profiling of the lipoprotein content were demonstrated. Additionally, the retention results could be used for calculation of diameter values. Analyses times ranged from 20 minutes to under an hour and plasma samples could be analyzed without sample pretreatment. Retention characteristics were well behaved as evidenced by the close agreement of calculated and literature Stokes diameter values. Distinctly different lipoprotein profiles were obtained for plasma samples of different individuals, suggesting possibilities for both clinical and research applications.

Table 1. Calculated protein and lipoprotein Stokes diameters using retention data from flow FFF analyses. Carrier was phosphate buffer saline at pH 7.4, V_x = 9.9 ml/min, V_{ch} - 3.1 mL/min. (adapted from reference 12.)

	Albumin	γ-globulin	HDL_3	HDL_2	LDL
Molecular Weight X 1000	66	158	150	300	3000
t_r (min) peak maximum	4.5	6.9	6.0	9.0	17.5
R retention ration	0.72	0.047	0.054	0.036	0.018
Diameter (nm) calculated	7.0	10.7	9.3	14.0	27.2
Diameter (nm) literature	7.4	11	8.5	12	25

Commercial Potential for the Proposed Lipoprotein Analyzer

The proposed flow FFF methodology is capable of high resolution measurements and is bio-compatible for analysis of biological macro-molecules ranging from proteins up to chromosomes and cells. Thus this instrumentation is potentially the most effective tool for direct determination of the entire subfraction set of the lipoprotein profile. The preliminary results in the study of a lipoprotein standard and a total plasma lipoprotein cholesterol sample showed excellent separation of HDL., LDL, VLDL, and other subfractions of the lipoprotein profile.

Use of the flow FFF method provides direct separation/characterization of the lipoprotein profile based on differences in physical size/molecular weight. The use of expensive enzyme

reagents is not required. In addition, the separated lipoprotein fractions maintain their original physical properties and so may be collected for further characterization and clinical studies. Other advantages of the proposed flow FFF technique are:

1) high accuracy and good reproducibility within +/- 3% CV.
2) rapid analysis within 20 minutes.
3) easily automated instrumentation and procedure.
4) inexpensive equipment ($30,000), operation and maintenance costs.
5) small sample size requirement: only 10 uL total blood plasma is needed for the lipoprotein profile test in comparison to the 7000 uL needed for the enzymatic reaction procedure.
6) cost per analysis is expected to be lower than the current clinical procedure due to the savings in enzymatic chemical usage and the use of less expensive equipment.

The magnitude of the need for screening serum cholesterol levels is immense and so creates an enormous market potential. As noted in this proposal, there are approximately 15,000 potential clinical laboratories which could become users of the FFF serum lipoprotein cholesterol analyzer. The potential sales could reach an estimated $22.5 million annually.

C. Relevant Experience

The principal investigator for the proposed research project will be Dr. Jane E. Smithe. Dr. John Jones will assist the project as co-investigator. Drs. Charles Pierce and William Little will participate as consultants. A post-doctoral fellow will also participate in the project to execute experimental milestones. Engineering assistance will be provided by Mr. Andrew Summer. Mr. Summer has a B.S. degree in physics. The qualifications of the investigators are listed below.

Principal Investigator

Dr. Smithe has 12 years experience with field-flow fractionation, as demonstrated by the publications and manuscripts listed on page 5. She graduated magna cum laude from Texas Lutheran College in 1979 with a B.S. degree in chemistry. In 1987 she graduated with a Ph.D. from the research group of Dr. Charles Pierce. The title of her thesis was “The Optimization of Sedimentation Field-Flow Fractionation for the Analysis of River Water Colloids.” The major focus of her work involved the study of electrostatic and Van der Waals interactions between colloidal particles and between particles and surfaces. During her graduate research period, she spent 8 months in the Water Research Center in Melbourne, Australia setting up instrumentation and training personnel.

After graduation, Dr. Smithe went to the analytical laboratories of Procter and Gamble in Cincinnati. There she had responsibilities in the separations, microscopy, and particle characterizations groups. With the aid of a summer undergraduate research student, she investigated the stability of oil-in-water emulsions using sedimentation field-flow fractionation.

In 1990, Dr. Smithe returned to Salt Lake City to manage the applications laboratory of Analytical Products, Inc. She consults with customers for their best use of field-flow fractionation techniques. She was involved also with designing the prototype Flow Field-Flow Fractionator and the High Temperature ThFFF Polyolefin Analyzer.

Co-Investigator

Dr. John Jones is the president and chief executive officer of Analytical Products, Inc. He has 20 years of industrial experience in product development and in marketing of new technology. Since 1966 he has been actively involved with the research, development, and marketing of many GC, HPLC, SFC, and capillary zone electrophoresis products.

Dr. Jones is one of the pioneers in field-flow fractionation, micro-HPLC, capillary column GC, and unified chromatography. His experience with the DNA sequencer in instrumentation design is important for the proposed Phase I studies. Dr. Jones was the first research scientist to obtain results in both flow and sedimentation field-flow fractionation. He is a consulting editor for the LC/GC Magazine, a member of the editorial board for the Journal of Liquid Chromatography, and is on the editorial advisory board of the Journal of Micro-Column Separation. Dr. Jones will contribute to the innovation, engineering, and development of the instrumentation of the Phase I proposal.

Post-Doctoral Fellow

Dr. Mary Yang has been extensively involved with biological applications of flow FFF since 1990. She is the author or co-author of several papers and presentations involving FFF. She has specifically dealt with lipoprotein samples and is responsible for the preliminary results discussed in the proposal. Her direct experience with lipoproteins and biological samples in general will be an asset to this project.

Dr. Yang's doctoral work involved HPLC, electrophoresis, and traditional separation and identification procedures as applied to the field of photochemistry. During her years as graduate, she was awarded two honors for excellence in graduate work.

D. Experimental Design and Methods

The primary goal of this research is targeted towards demonstrating the feasibility of a commercial adaptation of flow FFF technology for routine analysis of lipoproteins. This commercial development requires optimization of the instrumentation and analytical methods, and ultimately development of user friendly software to streamline and automate the analysis and data presentation. The criteria that must be met are:

1. The experimental method must be reliable, reproducible, and rugged, with acceptable recovery rates and minimal dilution effects.
2. Experimental conditions must be gentle so as to maintain the structural composition of the lipoprotein fractions.
3. Channel materials must be optimized for maximum operable channel lifetime.
4. Any chemical interaction of the membrane with the lipoprotein fractions must not influence the results.
5. Long-term chemical compatibility of the membrane with the carrier medium must be established.

Experimental Goals

Specific objectives for this Phase I proposal have been identified:

1. Evaluate the performance of several types of membranes including regenerated cellulose, and polypropylene for sample recovery, longevity, and analysis reproducibility. Test for interferences due to different sample preparation methods.
2. Optimize analysis conditions for separation of HDL, LDL, and VLDL components of blood plasma. Further optimize analysis conditions for high resolution separation of the sub-species of these fractions. Optimization parameters to include analysis time for acceptable sample throughputs, and resolution for accuracy of quantitation.
3. Construct several flow channels and evaluate system-to-system reproducibility, reliability, and longevity.
4. Establish accuracy of the method by comparison of the proposed Lipoprotein Analyzer results with ultracentrifugation results.
5. Develop user friendly software for automated, push-button analysis using the methods developed in Aim 3. (See below)
6. Plan Phase II development with the establishment of critical engineering milestones.

Experimental Design and Methods

The following experimental design and procedures are considered to be the most effective steps in pursuing our research and development effort towards the objectives given previously:

Aim 1. Evaluation of Membrane Performance

a. A test channel system will be set up for systematic studies and comparison of the physico-chemical properties of the various commercially available membranes. The following test protocol will be followed:

1. Surface uniformity and smoothness in both dry and wetted states.
2. Mechanical strength and pressure requirements for crossflow flux.
3. Porosity, pore size distribution, and molecular weight cut-off range.
4. Bio-compatibility of the membrane with blood plasma and lipoprotein samples.
5. Surface adsorption and charge properties.
6. Solvent compatibility and pH application range.
7. Lot-to-lot variations of membrane materials.
8. Long term performance

b. Test probes for sample adsorption and recovery studies will include standard proteins such as albumin and gamma-globulin, lipoprotein fractions, and blood plasma.

c. Test results will be compiled for easy identification of key control parameters and for definition of optimization steps.

Aim 2. Optimize Experimental Conditions for Analysis

a. Optimize cross flow and channel flowrate for separation efficiency, sample throughput, % sample recovery, and minimal dilution effects. Separation efficiency to balance resolution with analysis time.

b. Investigate the reliability of field programming for maximal sample recovery and minimal analysis time.

c. Develop optimal method for separation of basic HDL, LDL, and VLDL fractions as well as separate methods for specific separation of sub-fractions of each group.

d. Test probes to include standard proteins and purified lipoprotein fractions obtained using ultracentrifugation procedures.

Aim 3. Comparison of System-to-System Reliability

a. Construct three flow FFF channel systems using optimized membrane materials determined by Aim 1.

b. Analyze lipoprotein and blood plasma samples using optimized experimental conditions defined by Aim 2.

c. Compare results of the three flow channels for system-to-system reproducibility. If not acceptable, investigate channel system for design flaws affecting performance and revise channel accordingly. Recheck optimization as defined by Aim 2.

Aim 4. Establish Accuracy of the Quantitation of the Lipoprotein Fractions

a. Consult with industrial contacts and obtain sample prepared using varying techniques.

b. Analyze and quantitate lipoprotein fractions using optimized experimental methods as determined by Aim 2.

c. Tabulate and compare results for accuracy and interferences due to sample preparation techniques.

Aim 5. Develop Engineering Specifications for Software

a. Determine user inputs and specifications needed for routine analyses using methods determined by Aim 3.

b. Determine instrument interface needed for coupling with automatic sample injector.

c. Consult with software engineers for Phase II development of software as described by sections a and b above.

Aim 6. Establish Critical Engineering Milestones for Phase II Development

a. Conduct market research to determine user need and marketing specifications.

b. Propose engineering and marketing specifications for product development.

c. Establish product development milestones.

d. Prepare and submit final report with business for commercialization.

E. Human Subjects

1. Characteristics of the Subjects: Gender, Minority Status, and Age.

The target will be to select 20 normal human subjects, approximately 50% males and 50% females, and approximately 10% African-American, 10% Asian, and 10% Hispanic.The subjects will be between the ages of 18 and 55, and will be fasting for at least 12 hours prior to blood collection.

2. Sources of Research Materials.

Five ml blood will be collected from the antecubital vein, or another arm vein if necessary, under sterile conditions into EDTA anticoagulant tubes; processing of blood is described on page 16.

3. Recruitment Plans and Consent Procedures.

Subjects will be recruited from among employees of the company and of the University of Utah. A standard consent form approved and employed by the University of Utah will be used.

4. Potential Risks.
The risk is minimal and consists primarily of the possibility of a slight, temporary bruise appearing around the venipuncture site.
5. Procedures for Protecting Against or Minimizing Potential Risks.
Blood collection will be performed by University of Utah technicians or other professionals experienced in this procedure who have been approved to perform it on patients at the University of Utah Medical Center.
6. Potential Benefits to the Subjects and to Humankind.
Potentially this project could benefit these subjects and humankind by resulting in the development of a more rapid and accurate method for lipoprotein analysis in the future.

F. Vertebrate Animals

(This sample application does not involve vertebrate animals. However, if vertebrate animals are involved in the proposal, the application must address the following five items.)
1. A detailed description of the proposed use of the animals.
2. A justification for the choice of species and number of animals to be used.
3. Information on the veterinary care of the animals.
4. An explanation of procedures to ensure that the animals will not experience unnecessary discomfort, distress, pain, or injury.
5. Justification for any euthanasia method to be used.

G. Consultants

The following persons will work as consultants and their assistance will be useful for the successful completion of the proposed project. A brief vita and/or letter of consent is provided separately.

A. Charles Pierce, Ph.D., Chemistry Department, University of Utah
Professor Pierce was involved in the development of the field-flow fractionation method. He will assist in the interpretation of experimental data and the design and development of the commercial Lipoprotein Analyzer.

B. William Little, Ph.D., Chemistry Department, University of Utah
Professor Little has been involved in the design and construction of most of the prototype field-flow fractionation devices used to develop the technology to its present status. He was involved in designing the commercial thermal sedimentation FFF instruments marketed by Analytical Products, Inc.. Dr. Little will be instrumental in adapting the research version of the flow FFF apparatus into a more rugged, user-friendly commercial instrument.

C. Ann Howard, Ph.D., Department of Internal Medicine, University of Utah
Since, 1984 Dr. Howard's research interests have included analyses of lipoproteins. She is the author or co-author of more than 15 research articles of which roughly one third involve the study of lipoproteins. Dr. Howard will provide valuable information as to interpretation of the lipoprotein fractionation data as well as sample preparation techniques.

H. Contractual Arrangements

No contractual arrangements are involved in this proposal.

I. Literature Cited

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Department of Chemistry
University of Utah
Salt Lake City, UT 84119

March 5, 1996
Jane E. Smithe, Ph.D.
Analytical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449

Dear Dr. Smithe:

This letter is to express my willingness to serve as a consultant on your NIH Phase I proposal, "Development of a Lipoprotein Analyzer Based on Flow FFF." I agree to participate for six days of consultation at a minimum rate of $200 per day.

Sincerely,

Charles Pierce, Ph.D.

Department of Chemistry
University of Utah
Salt Lake City, UT 84119

March 5, 1996

Jane E. Smithe, Ph.D.
Analytical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449

Dear Dr. Smithe:

This letter is to express my willingness to serve as a consultant on your NIH Phase I proposal, "Development of a Lipoprotein Analyzer Based on Flow FFF." I agree to participate for seven days of consultation at a minimum rate of $200 per day.

Sincerely,

William Little, Ph.D.

Department of Chemistry
University of Utah School of Medicine
Salt Lake City, UT 84119

March 5, 1996

Jane E. Smithe, Ph.D.
Analytical Products, Inc.
2320 West St., Suite 16
Salt Lake City, UT 84119-1449

Dear Dr. Smithe:

This letter is to express my willingness to serve as a consultant on your NIH Phase I proposal, "Development of a Lipoprotein Analyzer Based on Flow FFF." I will be happy to support the proposed project by assisting in the area of sample preparation and data interpretation. I agree to participate for two days of consultation at a minimum rate of $200 per day.

Sincerely,

Ann Howard, Ph.D.

Checklist

TYPE OF APPLICATION *(Check appropriate box[es].)*

☒ NEW application. *(This application is being submitted to the Public Health Service for the first time.)*

☐ REVISION of previously-submitted application number _____
(This application replaces a prior unfunded version of a new application.)

☐ CHANGE of Principal Investigator *(if applicable)*
Name of former Principal Investigator _____

1. ASSURANCES/CERTIFICATIONS

The assurances/certifications set forth below are made and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (small business concern) on the FACE PAGE of the application. Descriptions of Individual assurances/certifications are found in application instructions under "Checklist." If unable to certify compliance with any item, provide an explanation and place it after this page.

● Human Subjects; ● Vertebrate Animals; ● Debarment and Suspension; ● Drug-Free Workplace; ● Delinquent Federal Debt; ● Research Misconduct; ● Civil Rights (Form HHS 690); ● Handicapped Individuals (Form HHS 690); ● Age Discrimination (Form HHS 690).

2. PROGRAM INCOME *(See discussion in application instructions under "Checklist.")*

All applications must indicate *(Yes or No)* whether program income is anticipated during the period for which grant support is requested.

☒ No ☐ Yes *(If "Yes," use the format below to reflect the amount and source(s) of anticipated program income.)*

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COSTS ***(See discussion in application instructions under "Checklist.")***

Insert the rate, if known. If the applicant organization does not have a currently negotiated rate with the Department of Health and Human Services (DHHS) or another Federal agency, it must estimate the amount of indirect costs allocable (applicable) to the proposed Phase I project. That amount should be inserted in the space provided below. The applicant organization should also be prepared to furnish financial documentation to support the estimated amount, If requested by the Public Health Service. An applicant organization may elect to waive indirect costs if it so desires.

☐ DHHS agreement, dated: _____ . _____ % salary and wages or _____ % Total Direct Costs.

☐ No DHHS agreement, but rate established with _____ , dated: _____

☐ Rate negotiable pending with the National Institutes of Health.

☒ Indirect costs allocate (applicable) to this Phase I project are estimated to be $ 32000 (40% of 80,000)

☐ No indirect costs requested.

4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workspace and/or promote the non-use of tobacco products or have plans to do so?

☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*

Principal Investigator (Last, first, middle): Smithe, Jane E.

Place this form at the end of the signed original copy of the application. Do not duplicate.

Social Security No. 111-11-1111

PERSONAL DATA ON PRINCIPAL INVESTIGATOR

The Public Health Service (PHS) has a continuing commitment to monitor the operation of its review and award processes to detect—and deal appropriately with—any instances of real or apparent inequities with respect to age, sex, race, or ethnicity of the proposed principal investigator.

To provide the PHS with the information it needs for this important task, complete the form below and attach it to the signed original of the application after the Checklist. Do not attach copies of this form to the duplicated copies of the application.

Upon receipt of the application by the PHS, this form will be separated from the application. This form will not be duplicated, and it will not be a part of the review process. Data will be confidential, and will be maintained in Privacy Act record system 09-25-0036, "Grants: IMPAC (Grant/Contract Information)." The PHS requests Social Security Numbers for accurate identification, referral, and review of applications and for management of PHS grant programs. Provision of the Social Security Number is voluntary. No individual will be denied any right, benefit, or privilege provided by law because of refusal to disclose his or her Social Security Number. The PHS requests the Social Security Number under Sections 301(a) and 487 of the PHS Act as amended (42 USC 241a and USC 288). All analyses conducted on the date of birth and race and/or ethnic origin data will report aggregate statistical findings only and will not identify individuals.

If you decline to provide this information, it will in no way affect consideration of your application.

Your cooperation will be appreciated.

DATE OF BIRTH (MM/DD/YY)	GENDER
2/10/46	[X] Female [ ] Male

RACE AND/OR ETHNIC ORIGIN *(check one)*

Note: The category that most closely reflects the individual's recognition in the community should be used when reporting mixed racial and/or ethnic origins.

[ ] ***American Indian or Alaskan Native.*** A person having origins in any of the original peoples of North America, and who maintains a cultural identification through tribal affiliation or community recognition.

[ ] ***Asian or Pacific Islander.*** A person having origins in any of the original peoples of the Far East, Southeast Asia, the Indian subcontinent, or the Pacific Islands. This area includes, for example, China, India, Japan, Korea, the Philippine Islands, and Samoa.

[ ] ***Black, not of Hispanic origin.*** A person having origins in any of the black racial groups of Africa.

[ ] ***Hispanic.*** A person of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race.

[X] ***White, not of Hispanic origin.*** A person having origins in any of the original peoples of Europe, North Africa, or the Middle East.

[ ] Check here if you do not wish to provide some or all of the above information.